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# Muscle Sympathetic Reactivity to Apneic and Exercise Stress in High-Altitude Sherpa

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## ABSTRACT

Lowland-dwelling populations exhibit persistent sympathetic hyperactivity at altitude that alter vascular function. High altitude populations, such as Sherpa, have previously exhibited greater peripheral blood flow in response to acute stress than Lowlanders which may be explained through lower sympathetic activity. Our purpose was to determine if Sherpa exhibit lower sympathetic reactivity to stress than Lowlanders. Muscle sympathetic activity (MSNA; microneurography) was measured at rest in Lowlanders (n=14; age=27±6yrs) at 344m and following a 8-9 days at 5050m. Sherpa (age=32±11yrs) were tested at 5050m (n=8). Neurovascular reactivity (ie. change in MSNA patterns) was measured during maximal end-expiratory apnea, isometric hand-grip (IHG; 30% maximal voluntary contraction for 2-minutes) and post-exercise circulatory occlusion (PECO; 3-minutes). Burst frequency (bursts/min), incidence (bursts/100HB), and total normalized SNA (au/min) was analyzed at rest, immediately prior to apnea breakpoint, and during the last minute of IHG and PECO. Vascular responses to apnea, IHG, and PECO were also measured. MSNA reactivity to apnea was smaller in Sherpa than Lowlanders at 5050m, though blood pressure responses were similar between groups. MSNA increases in Lowlanders during apnea at 5050m were significantly lower than at 344m ($P<0.05$), indicating possible sympathetic ceiling was reached in Lowlanders at 5050m. MSNA increased similarly during IHG/PECO in Lowlanders at both 334m and 5050m, and Sherpa at 5050m, while vascular changes (mean brachial arterial pressure, contralateral brachial flow/resistance) were similar between groups. Sherpa demonstrate overall lower sympathetic reactivity which may be a result of heightened vascular responsiveness to potential apneic stress at altitude.

## INTRODUCTION

The response to both acute (18, 21, 30, 31, 36) and chronic (e.g. altitude) (6, 13, 22, 25) reductions of oxygen availability in lowland dwelling populations is an increase in basal efferent sympathetic activity (SNA). This sympathoexcitation occurs in conjunction with local dilation to facilitate blood flow redistribution and oxygen delivery to critical tissues. Previous findings also show an augmented SNA response to exercise (i.e. increased sympathetic reactivity) under acute hypoxic conditions (18, 33) . Whether a similar potentiation occurs during chronic hypoxia is unknown. As Lowlanders exhibit heightened SNA at altitude, we propose a similar potentiation of sympathetic reactivity to additional stress. Furthermore, whether similar responses are evident in those exposed to long duration hypoxia, such as high altitude natives has not been studied. Nepalese/Tibetan Sherpa have resided at high altitude (>2500m) for thousands of years, allowing for unique evolutionary phenotypic adaptations under chronic hypoxia exposure. This includes not only cardiovascular adaptations that allow for increased oxygen delivery and metabolism at the local tissue (7, 9, 41) , but also overall lower SNA at rest compared to Lowlanders at altitude (35) . Limited data suggest that Sherpa exhibit an improved ability to increase systemic blood flow at altitude (9, 32) , while also showing greater capillary density within skeletal muscle (19) and improved ability to increase leg blood flow (32) . Given our previous findings have shown that Sherpa exhibit lower basal MSNA compared to Lowlanders (35) ; we hypothesize that these differences in vascular function between Lowlanders and Sherpa may also translate to lower sympathetic reactivity to acute apneic/exercise stress.

To address the hypothesis, we performed microneurographic recordings of efferent post-ganglionic nerve activity in Lowland dwellers at low (344m) and high (5050m) altitudes and in a group of native Sherpa at altitude (5050m). This was complemented with brachial ultrasonography to determine the effect of heightened sympathetic stress on vascular function during stress in Sherpa and acclimatized Lowlanders.

## METHODS

This study was carried out within the framework of the 2016 UBC Nepal Expedition to the Ev-K2-CNR Research Facility (5050m) (40) . Though participants took part in a number of independent investigations, experiments were organized to ensure no contamination between studies, and each study addressed distinct *a priori* research questions. Baseline demographics, cardiovascular characteristics and heart rate responses to apnea have been previously reported from Lowlanders and Sherpa at altitude (3, 38) , while metrics of basal SNA have also been previously reported by our group (35) . Therefore, basal SNA data is included as a complement for the novel independent analyses related to sympathetic reactivity.

### Study Participants

Fourteen Lowlanders (27±6yrs; 2 female) and ten Sherpa (32±11yrs; 0 female) from the Khumbu region of Nepal participated after providing informed written consent in their native language. Procedures were explained in Nepalese and English as needed, and were approved by the University of Alberta Biomedical Research Ethics Board, University of British Columbia Clinical Research Ethics Board, and Nepal Health Research Council. Participants were free of ventilatory, cardiovascular, metabolic, and neurological disorders as determined by a self-

reported health history questionnaire. Four Sherpa were self-reported smokers (0.4±0.7 pack years).

**Testing Location(s)**

The ascent profile and testing schedules are outlined in Supplemental Figures 1 (https://doi.org/10.6084/m9.figshare.8066717), 2 (https://doi.org/10.6084/m9.figshare.8066711), and 3 (https://doi.org/10.6084/m9.figshare.8066714.v1). Pre-expedition testing of Lowlanders (n=14) was performed at 344m (Kelowna, Canada). To match the ascent profile and acclimatization process between groups, Sherpa were flown to Kathmandu, Nepal (1400 m), where they resided between 5-15 days. Both Lowlanders and Sherpa flew to Lukla, Nepal (2840m) and followed a 9-10 day ascent. One lowlander was administered oral acetazolamide (half life - 4 hrs) and another was administered an intramuscular injection of dexamethasone (half life – 3 hours) following 4 days arrival at 5050m for the treatment of acute mountain sickness; however, both were tested after a 48-hour washout. Sherpa were not on any medication and were tested on days 1-3 following arrival at 5050m, while Lowlanders were tested between days 1-10 (Supplemental figure 2).

***Study Protocol***

Following instrumentation, basal SNA and cardiovascular function were measured during 10 minutes of quiet rest. Sympathetic reactivity was subsequently assessed using two protocols: 1) a volitional end-expiratory apnea at functional residual capacity (23) and 2) isometric hand-grip (IHG) performed for 2 min followed by 3 min of post-exercise circulatory occlusion (PECO). Prior to apnea, an investigator paced the participants' breathing (2-3 breaths) to maintain rate and

depth, while preventing hyperventilation. Participants were then instructed to "hold their breath for as long as possible. Participants performed IHG at 30% of their previously determined maximal voluntary contraction using handgrip dynamometer (Grip Force Transducer; ADInstruments, Australia). Immediately following 2 minutes of IHG, a manual blood pressure cuff was inflated (>200mmHg) for 3 minutes to stimulate post-exercise ischemia while the limb was relaxed. The apnea protocol always preceded IHG/PECO protocol.

**Experimental Measures**

All participants were tested in the supine position. ECG (Lead II) and the arterial blood pressure waveforms (finger photoplethysmography; Finometer Pro, Finapres Medical Systems, Netherlands) were collected continuously at 1 KHz (ADInstruments, Chart Pro v8.3.1, Australia). Heart rate (HR) was calculated from the ECG R-R interval. Beat-by-beat cardiac output (CO) was calculated using the Model Flow algorithm and used to calculate total peripheral resistance (TPR = MAP/CO). Beat-by-beat mean (MAP), systolic (SBP) and diastolic (DBP) pressures were calculated from the arterial pressure waveform that was calibrated against manual sphygmometry.

***Muscle Sympathetic Nerve Activity***

Microneurography was used to directly measure efferent muscle sympathetic vasomotor nerve activity (MSNA) (11, 35, 37) . A tungsten microelectrode (200μm diameter, 35 mm long, tapered to a 1-5 μm uninsulated tip) was inserted percutaneous into the peroneal (common fibular) nerve, with an uncoated tungsten reference electrode inserted subcutaneously 1-3 cm from the recording site. The recording electrode was manipulated until a pulse-synchronous bursting pattern was

identifiable in response to apnea but not a loud noise (4) . The raw MSNA signal was amplified (1000x pre-amplifier and 100x variable gain isolated amplifier), band pass filtered (700-2,000Hz), rectified, and integrated (decay constant 0.1s) to obtain a mean voltage neurogram (model 662C-3; Iowa University Bioengineering; USA). Both raw and integrated signals were sampled at 10 KHz (ADInstruments, Chart Pro v8.3.1; Australia).

***Vascular Ultrasonography***

Of the 22 participants tested, ultrasonograhy was successfully obtained a subset of Lowlanders (n= 8) and Sherpa (n = 4). Ultrasonography was used to measure brachial artery (BA) diameter, Doppler velocity ($BA_V$), and flow ($BA_F$) in the non-exercising arm at baseline and during the IHG/PECO protocols (12 ~MHz linear array transducer; Vivid Q, GE Healthcare). Probe insonation-angle was kept constant (60°) across all tests. Video capture was used for recording vessel diameter (DVI2USB3.0; Epiphan Systems, Canada) and was stored off-line in audio video interleave format for future analysis with edge detection software (Brachial Analyzer, Medical Imaging Applications, USA). Image analysis of data was performed at 30Hz following visual confirmation (SAB) of the region of interest to ensure clearly distinguishable lumen walls. BA flow velocity waveforms were converted from Doppler audio signals (qDAT; Penn State, USA) (14) and stored offline at 1 KHz (ADInstruments, Chart Pro v8.3.1).

**Data and Statistical Analysis**

Resting MSNA and cardiovascular data was averaged over ~10 minutes. MSNA bursts were identified using a semi-automated detection algorithm (Chart Pro 8.3.1) and confirmed by a

trained observer (SAB). Resting MSNA was quantified as burst frequency (bursts/min), incidence (bursts/100 HB), and normalized burst amplitude (% of maximal burst size at baseline) and area (area under the curve, [au]). For the apnea protocol, MSNA and cardiovascular data were analyzed from the final 10 cardiac cycles prior to volitional breakpoint. Cardiovascular post-apnea nadir ($S_pO_2$, HR) and peak (SBP, DBP, MAP) responses were obtained in 10-15 seconds post breakpoint. MSNA bursts during the apnea were calculated as the burst area (area under the curve, [au]) during the last 10 cardiac cycles prior to volitional breakpoint. Burst area was normalized SNA (au/min) during baseline and apnea to account for variations in cardiac cycle length and burst width (3) . In addition, the average likelihood (%) of a burst occurring during a given cardiac cycle for apneas was calculated across participants. Sympathetic reactivity to apnea was assessed as the increase in normalized burst area was compared between baseline and end-apnea. MSNA (frequency, incidence and normalized amplitude) and cardiovascular data (HR, blood pressure, TPR, and $BA_F$) were averaged during the last minute of both IHG and PECO. Sympathetic reactivity was assessed as the increase in MSNA from baseline to IHG and PECO. Finally, an indirect measure of neurovascular transduction was performed to determine the translation of sympathetic outflow on vascular outcomes (delta change in TPR over the delta change in delta burst frequency [au]) across groups during the IHG/PECO protocols.

Results are reported as mean ± standard deviation (SD) with the exception of baseline burst amplitude, which is reported as median ± interquartile range (IQR) to account for differences in amplitude distribution between conditions. Multiple comparisons were assessed for all measurements using pre-planned contrasts of Lowlanders from low to high altitude (paired t-tests), and Lowlanders to Sherpa at high altitude (unpaired t-tests) with an adjusted alpha ($\alpha'$)

value corrected for multiple comparisons ($c$). This was performed by adjusting the *a priori* alpha ($\alpha$, 0.05) using the experiment-wise error rate ($\alpha_e$) (3, 15) :

$$\alpha' = \frac{\alpha_e}{c}$$

$$\alpha_e = 1 - (1 - \alpha)^c$$

For normalized bursts amplitude, and vascular responses during baseline, IHG, and PECO protocols, a two-way repeated measures ANOVA compared the main and interaction effects in the distributions between conditions. Statistical analysis for normalized total peak SNA and change in mean arterial pressure was performed via one-way ANOVA between Lowlanders at low altitude, Lowlanders at High Altitude. To address the potential effect of duration at altitude on resting MSNA; a secondary analysis via Pearson's moment correlation analysis of dependent variables was performed in this study. Finally, ANCOVA analysis was used to control for duration at altitude. All statistical analyses were performed using SigmaPlot 13 (Systat Software, Chicago, IL).

**RESULTS**

Fourteen lowlanders were successfully tested at 344m and again at 5050m. Two Lowlanders reported having mild acute mountain sickness (AMS) on the day of testing (Lake Louise scores of 3). However, the data from these two subjects were comparable to the averaged responses and therefore included in the main analyses. Eight of the ten Sherpa who descended to Kathmandu were successfully tested at 5050m. No relationship was shown in this study between duration after arriving at 5050m and resting MSNA in either Lowlanders (Burst Frequency $r^2$= 0.160, p= 0.065) or Sherpa (Burst Frequency $r^2$ = 0.001, p=0.937). Baseline cardiovascular and

autonomic characteristics for both Sherpa and Lowlanders are listed in Table 1. SBP, DBP, MAP, CO, TPR, and SpO2 were not different between lowlanders and Sherpa at 5050m

**Baseline Neurovascular Characteristics in Sherpa and Lowlanders**

Resting MSNA values for Sherpa and Lowlanders have been reported previously reported (35) , but are displayed in Tables 1 for completeness. Briefly, Lowlanders burst frequency tripled (11 ± 5 bursts/ min to 30 ± 7 bursts/ min; $p<0.001$) while burst incidence doubled (25 ± 13 bursts/100 HB to 50 ± 15 bursts/ 100 HB; $p<0.001$) following several days at high altitude. At 5050m, Sherpa had a lower burst frequency (23 ± 11 bursts/min; $p<0.05$) and incidence (30 ± 13 bursts/100 HB; $p<0.05$) compared to Lowlanders at 5050m. At altitude the distribution of normalized burst area was also shifted towards larger sized bursts in Lowlanders, with the burst amplitude distributions in the Sherpa being similar to that of Lowlanders at 5050m. After taking into account differences in both frequency and amplitude distribution, total basal sympathetic activity was similar in Sherpa (1103 ± 520 au/min) and Lowlanders at 5050m (1320 ± 520 au/min; $P=0.385$) with both being higher than observed in lowlanders at low altitude (451 ± 206 au/min; $p<0.05$).

**Sympathetic Reactivity to Apnea in Lowlanders and Sherpa**

Sympathetic neurovascular reactivity during apnea was assessed in 14 Lowlanders at low and high altitude, and 8 Sherpa assessed at 5050m. At low altitude, Lowlanders had an apnea duration of 30.4 ± 11.1 s (range 15-74s) which was reduced to 15.4 ± 5.3 s (range 9-27s) ($P<0.001$) at 5050m. Lowlanders $SpO_2$ nadir post-apnea was 78 ± 7%. Sherpa apnea duration (15.8 ± 2.6; Range 12-19s; $P = 0.84$) and saturation (75 ± 5%; $P=0.329$) post-apnea were not different to that of Lowlanders.

Apnea across all groups and conditions produced a very robust increase in MSNA driven by changes in both burst occurrence and burst area (Figures 1-3). Apnea at low altitude was associated with a significant increase in MSNA in Lowlanders (normalized total area +31359 ± 30383 au/min compared to baseline; $P<0.01$). While apnea at altitude resulted in the largest burst augmentation and increase in burst occurrence (Figure 2), this occurred over a longer time due to a previously reported bradycardia response (3) Thus, the au/min response to apnea tended to be less at high altitude (+17711 ± 11018 au/min) versus low altitude (+31359 ± 30434 au/min; $p=0.063$). Interestingly, 5 out of the 14 Lowlanders had "prolongation" of sympathetic bursts during apnea at high altitude that did not represent normal burst firing characteristics. More specifically, the cyclical modulation of efferent bursts activity was altered at 5050m such that bursts became broader and less peaked, encompassing a larger portion of the cardiac cycle (Figure 1). In addition, there was a brief period post-apnea where no bursts occurred (approx.5-10 seconds) immediately following volitional breakpoint, after which 'characteristic bursts' returned to those observed pre-apnea. In contrast, Sherpa sympathetic responses to apnea (+7708 ± 4312 au/min) were significantly lower than Lowlanders at low altitude ($P=0.048$) and at 5050m ($P=0.027$). Additionally, there were no observed cases of "prolonged" bursts in Sherpa neurograms.

Apnea resulted in a significant increase in mean blood pressure in all three groups; 34 ± 13 mmHg in Lowlanders at low altitude, 35 ± 20 mmHg in Lowlanders at 5050m and 23 ± 8 mmHg in Sherpa at 5050m (all $P<0.01$ with respect to baseline) (Figure 3). The increase in MAP associated with apnea was smallest in Sherpa ($P=0.028$ when compared to Lowlanders at 334m). When these responses were considered together Sherpa had higher vascular responsiveness to sympathetic activation during apnea ($3.70 \pm 1.90$ mmHg/au/min x $10^{-3}$)

compared to Lowlander at low (1.84 ± 1.17 mmHg/au/min x $10^{-3}$, P<0.01) but not high (2.62 ± 1.81 mmHg/au/min x $10^{-3}$, P=0.227) altitude.

**Sympathetic and Vascular Reactivity to Isometric Hand Grip and Post Exercise Circulatory Occlusion**

Sympathetic neurovascular reactivity during the IHG/PECO protocols was assessed in 14 Lowlanders at low and high altitude, and was successfully collected in 6 Sherpa 5050m. At altitude, Sherpa exhibited an overall lower burst frequency, incidence and total MSNA during the BL, IHG and PECO compared to acclimatized Lowlanders (Figure 4, each P<0.001). During IHG burst frequency (+17 ± 9, +18 ± 13, and +16 ± 12 bursts/min; all P<0.001), burst incidence (+14 ± 15, +5 ± 15, and +13 ± 13 bursts/100 HB; all P<0.001) and total MSNA (+1429 ± 893, +1247 ± 1178, and +1827 ± 1361 au/min; all P<0.001) were elevated significantly in Lowlanders at 344m and 5050m, and Sherpa at 5050m respectively (Figure 4). No further increase in burst frequency or total MSNA occurred between IHG and PECO, although burst incidence climbed due to the concurrent return of heart rate to baseline during PECO (Figure 5). The increase in MSNA occurring with IHG/PECO was not different between groups.

While Sherpa tended to have an overall lower blood pressure (Main effect of group P=0.32; P=0.27 versus Lowlanders in Kelowna; P=0.93 versus Lowlanders at 5050m), the absolute change in blood pressure response to IHG and PECO were similar between Sherpa (+15± 4 and +12± 6 mmHg) and Lowlanders (+15± 6 and +12± 8 mmHg) at 5050m (Figure 5; P=0.829 for IHG, P=0.778 for PECO). The change in total peripheral resistance was also similar between Sherpa (+2.6± 2.8 and +2.9± 2.9 mmHg/L/min) and Lowlanders (-0.7± 1.9 and

+0.6± 1.7 mmHg/L/min) at 5050m (P=0.791 for IHG, P=0.836 for PECO). Thus, unlike the apnea protocol we did not detect significant differences in neurovascular reactivity during IHG / PECO stress with altitude (in Lowlanders) or between groups (at 5050m). Indirect transduction analysis revealed that Sherpa showed greater vascular transduction than Lowlanders at 344m (+151.7± 73.1 versus -74.7 ±184.7 au; P=0.015), but not Lowlanders at 5050m (0.9± 262.0 au; P=0.198), during the IHG protocol. However, the transduction response was similar across all groups during the PECO protocol, respectively (+178.2 ± 142.5; +214.0± 131.0 [ P=0.657 versus Sherpa]; and +7.5 ± 279.6 [P =0.187 versus Sherpa] au).

**Forearm Blood Flow Reactivity in Lowlanders and Sherpa**

Brachial artery blood flow was successfully analyzed in a subset of 8 Lowlanders and 4 Sherpa during the HG/PECO protocol (Figure 6). Basal forearm blood flow was not different between Lowlanders at 344m (46± 39 mL/min), 5050m (22± 42 mL/min), or Sherpa at 5050m (56± 24 ml/min) (Main effect of Group, P=0.080). Both IHG and PECO saw no change in contralateral brachial blood flow for Sherpa (+8± 23, P=0.836 and +11± 38 mL/min, P=0.522 respectively) and Lowlanders (+29± 25, P=0.624 and +22± 42 mL/min, P= 0.644 respectively) at 5050m. The HG and PECO protocols did not result in a change in contralateral brachial resistance for Sherpa (-0.3± 1.1, P=0.879 and 0.1± 0.8 mmHg/mL/min, P=0.987 respectively) and Lowlanders (-0.6± 1.0, P=0.945 and -0.2± 0.9 mmHg/mL/min, P=0.898 respectively) at 5050m, similar to blood pressure results (above). However, analysis of conductance proved more sensitive, indicating significant main effect for brachial artery conductance (P=0.049), with Lowlanders having a higher brachial artery conductance at altitude than Sherpa (P=0.047, Figure

6). Nonetheless, there remained no absolute change in conductance during IHG and PECO for either Sherpa (-0.1± 0.3 and -0.1± 0.5 mmHg/mL/min) or Lowlanders (+0.2± 0.3 and +0.1± 0.5 mmHg/mL/min) at 5050m.

## DISCUSSION

This study demonstrates that sympathetic neural activation (i.e. increases in MSNA) in response to acute apneic stress appears lower in Lowlanders following acclimatization. However, this is due to the unique nature maximal volitional apneas. Nonetheless, Sherpa had a lesser response compared to acclimatizing Lowlanders at 5050m and Lowlanders at low altitude (334m). This lower MSNA response in Sherpa was offset by a greater vascular reactivity to sympathetic activation. During isometric hand-grip and post-exercise circulatory occlusion, sympathetic activation was observed to be much lower than the apneic stress, and no differences were noted between groups with respect to sympathetic activation or vascular responses.

### *MSNA responses to apneic and hand-grip / post-exercise circulatory occlusion stressors*

Apnea proved to be a significant sympathetic stressor, both at low and high altitude. Although acclimatized Lowlanders demonstrated 100% burst occurrence and the greatest burst augmentation (i.e. increase in burst area), normalized total activity remained lower than that measured at low altitude. This was due to a previously reported bradycardia [16] that limits burst frequency. Thus, an apparent "sympathetic ceiling" may be reached during apnea in chronic hypoxic conditions. Despite similar apnea durations and desaturation in Sherpa compared to acclimatized Lowlanders, the Sherpa demonstrated a lower burst occurrence, lower burst

augmentation and a lower total MSNA response to apnea. Importantly, this occurred without an apparent limitation on burst frequency (i.e. no bradycardia). These data indicate that Sherpa are less responsive to apneic stress under the same hypoxic conditions as acclimatized lowlanders. This also suggests that Sherpa have a greater functional sympathetic reserve, whereby they may theoretically be able to increase MSNA more than observed in the current study. In contrast to apneic stress, the MSNA response to the IHG/PECO protocol was appreciably smaller (although not assessed statistically). Although a main effect of group was present, with acclimatized Lowlanders having the highest activity, all three groups exhibited similar increases in MSNA burst frequency, incidence, and total activity during IHG/PECO.

Previous studies in Lowlanders under acute hypoxic exposure demonstrate further MSNA potentiation during dynamic exercise compared to normoxia. (18, 34) Since the ascent to altitude causes further reductions in oxygen availability to the local tissue alongside concurrent MSNA augmentation, we hypothesized that altitude would also be associated with an augmented SNA response to IHG and PECO. However, the lack of difference in the response at low and high altitudes demonstrate that chronic hypoxic stress compounded with further metabolic activation (albeit in one isolated limb during an isometric exercise) does not alter the MSNA response. Therefore, MSNA reactivity to metaboreflex stress appears preserved in acclimatized Lowlanders. We believe that this may be explained through several myogenic adaptations during acclimatization that favor anaerobic metabolism under chronic hypoxia exposure. With long-term exposure to altitude there has been previously noted reductions of muscle oxidative capacity (16) associated with muscle atrophy and catabolism (2) in addition to a shift away from FA enzyme oxidation during rest and exercise (29) . These potential adaptations during

longer periods of residency at altitude would improve anaerobic metabolism, reduce metabolic strain and overall sympathetic activation compared to exertion during an acute period of hypoxia exposure. These adaptations may be time dependant, with shorter periods of exposure not appearing to show any significant changes in skeletal muscle function or morphology (26) . Therefore, any potential myogenic adaptations that may exist in the first week of acclimatization for Lowlanders does not appear to directly affect efferent sympathetic outflow. Previous data have indicated lower mitochondrial density (19) , improved ATP to $O_2$ yield and greater energy production at a lower oxygen cost in Sherpa (17) . Sherpa have also previously demonstrated increased ability to augment femoral blood flow velocity post-circulatory occlusion compared to Lowlanders (32) . For these reasons, we hypothesized that Sherpa would have a lower MSNA response to the IHG/PECO protocol. Counter to this hypothesis, we observed that Sherpa had a similar increase in MSNA during IHG/PECO. It is worth noting that MSNA was lower in Sherpa compared to acclimatized Lowlanders through baseline and IHG/PECO. Thus, the above noted mechanisms could still be involved in shifting the MSNA relationship lower, but keep the same gain of the response to metabolic stress.

**Neurovascular Reactivity between Lowlanders and Sherpa at Altitude**

During both apnea and IHG/ PECO reactivity protocols, there was no noted difference in pressor responses to apnea or IHG/PECO in Lowlanders at low or high altitudes, with exception to a lower TPR response under the PECO condition at altitude compared to sea level. Thus, the current study demonstrates that overall cardiovascular reactivity was preserved during altitude acclimatization 5050m. From the findings of both reactive conditions the vasoconstrictive response was greater in Sherpa during apnea, but not IHG/PECO, which was shown in the

indirect transduction findings. We postulate two explanations for this disparity: 1) the magnitude of the response to apnea was much larger than during the IHG/PECO protocol, and thus may have been more robust for identifying differences in pressor responses between groups; and 2) the lack of change in mean arterial pressure is representative of total systemic changes in TPR and cardiac output. Thus, the modest increase in MSNA may not have had a significant influence on altering mean arterial pressure. The subset of data evaluating brachial artery blood flow support this hypothesis. Although our data do not support differences in the cardiovascular response to small muscle mass recruitment in Sherpa, we acknowledge previous data which suggests other cardiovascular adaptations in this population. Sherpa have previously been shown to exhibit greater capillary density within skeletal muscle (19) and improved ability to increase leg blood flow following 2 minutes of circulatory occlusion (32) . Ezurum *et al.* (7) subsequently demonstrated that Tibetans have higher resting forearm blood flow and circulating NO by-products. These previous data support an improved dilatory capacity, but our data also support a lower resting sympathetic activity, a greater sympathetic reserve and greater vascular sensitivity to higher levels of sympathetic activity. Together, this may serve as an important control mechanism for redirected blood and oxygen during stress. Thus, Sherpa appear to have developed improved cardiovascular efficiency that does not rely to the same extent on sympathetic hyperactivity relative to acclimatized Lowlanders. Whether this is expressed through other high altitude populations remains to be determined.

**Considerations**

An interesting finding for Lowlanders was the lower total absolute MSNA responses to apnea at high altitudes, despite basal MSNA being augmented at 5050m. As the apnea duration

was shorter, in combination with a lower post-breakpoint $SpO_2$ (indicative of an increase chemoreceptor activation) (8, 39) , it can be argued that apnea at altitude is a greater sympathetic stressor than it is at low altitude. However, the concurrent bradycardia which we have previously reported on (3) likely limited sympathetic activation and could explain the appearance of abnormal MSNA burst patterns at 5050m. This demonstrates a potential sympathetic "ceiling effect" may have developed in Lowlanders, where further stress does not produce additional MSNA activation. In other words, there is less MSNA reserve available for responding to acute stress at altitude. MSNA outflow is limited to an individuals' respective cardiac cycle, where a finite degree of sympathetic augmentation can occur during each R-R interval (4, 24) . Whether Sherpa truly have additional MSNA reserve available during apnea, or simply reached their own respective sympathetic ceiling, cannot be confirmed due to us being unable to obtain sympathetic reactivity in Sherpa at Kathmandu. However, the absence of abnormal burst pattern and a lower average incidence of bursts in the cardiac cycles preceding break-point supports this premise.

Though we report that MSNA is lower across both basal and reactivity conditions for Sherpa, the specific mechanism that contributes to this overall lower MSNA response has not yet been determined. During the transition between acute to chronic hypoxia exposure there is an apparent time-dependant sensitization for the peripheral chemoreceptors that results in progressively heightened MSNA (5) . This is supported by a higher basal MSNA previously observed by Hansen and Sander (13) and further confirmed by Lundby *et al.* (22) . If this were true then it could be argued that attenuated chemoreflex sensitivity in Sherpa should explain their lower basal MSNA. However, indirect measures of chemoreceptor sensitization do not appear to

387 explain the differences in MSNA between groups, as the current consensus (including more
388 recent publishing from this expedition) demonstrate similar hypoxic ventilatory responses
389 between acclimatized Lowlanders and Sherpa (1, 3, 10) . Though we do not believe the
390 peripheral chemoreflex is the primary driving mechanism for why sympathetic reactivity differs
391 between groups; we cannot completely exclude it given that ventilatory and sympathetic
392 responses may differ when under chemoreflex engagement (20) . Regardless, the lower MSNA
393 noted for Sherpa is likely attributed to a combination of other reflexes. These may include
394 differences in long-term potentiation of central regulatory mechanisms, central resetting, or
395 baroreflex-mediated changes between Lowlanders and Sherpa (12, 28, 42) ; the latter is
396 addressed in a parallel paper by this research group (35) .
397
398 **Limitations**
399 As testing of Lowlanders and Sherpa occurred during the initial 10 days of being at
400 5050m, we acknowledge that the findings within this study may be in part influenced by the
401 respective date individuals were tested at 5050m. As previously stated we assessed the potential
402 covariate of duration through Pearson's correlation and follow up ANCOVA analysis, where
403 neither burst frequency or incidence reached significance($P=0.065$ and $p=0.937$). However,
404 when examining baseline sympathetic function, and both sympathetic and vascular reactivity
405 between groups, there was no relationship following correction for the day they were tested at
406 5050m. We acknowledge the lack of relationship between duration of residency and resting
407 MSNA may be due to our small sample size. Although there may exist a gradual increase in
408 sympathetic activity following prolonged durations at altitude, this does not appear to have
409 affected our results of the current study. Furthermore, Lundby *et al.* (22) also showed that

MSNA was similar in acclimatized Lowlanders between days 10 and 50 at 4100 m. MSNA therefore does not appear to increase further following several days at altitude, though the exact period is currently undefined. However, we also acknowledge that degree of sympathetic activation may be dose dependant with regards to the specific severity of hypoxic exposure (31) . Whether MSNA shows further augmentation over the span of days-weeks should be considered for future studies.

The use of voluntary apnea is a simple model of assessing muscle autonomic reactivity as it evokes both a quick and large sympathetic response. However, apnea tolerance can be objectively difficult to assess as duration can be affected by several factors including previous repetitive practice overall tolerance to apneic stress between individuals (27) . This raises the question of whether Sherpa truly demonstrated a maximal apnea at altitude. However, all experimental procedures and manoeuvres were explained to Sherpa in Nepali and trials were repeated if there was any confusion. In addition, Sherpa had both a similar apnea duration and drop in $SpO_2$ to that of Lowlanders.

**Perspectives and Significance**

The current study demonstrates several novel findings, including that: 1) overall resting MSNA activity and reactivity to apneic stress is lower in Sherpa at altitude; 2) sympathetic reactivity to stress in acclimatized Lowlander appears to have be finite given the findings of a potential “sympathetic ceiling”; and 3.) overall vascular reactivity was preserved during altitude acclimatization 5050m in both Lowlanders and Sherpa, demonstrating that vascular responsiveness to sympathetic activation may differ between groups. However, the specific mechanisms that governs these differences between acclimatized Lowlanders and Sherpa is

uncertain. We propose that the observation of an apparent sympathetic plateauing observed in Lowlanders indirectly supports a potentially greater sympathetic reserve at altitude for Sherpa, though our findings from Kathmandu are underpowered to confirm this. Altered vascular responsiveness in Sherpa may be a beneficial adaptation to generational residency that prevents chronic hypertensive states while allowing greater vascular control when necessary to increased physical demands at altitude. In general, further research is needed in order to delineate underlying mechanisms that underpin autonomic and neurovascular control at high altitude in native Sherpa.


## ACKNOWLEDGMENTS

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## DISCOLSURES

None

## FIGURE CAPTIONS

**FIGURE 1:** Integrated neurogram demonstrating sympathetic activity during baseline (left) and reactivity during apnea (right). Apnea shows last 10 cardiac cycles obtained prior to volitional breakpoint. *Panel A,B,C.* Example of Neurogram from the same male at 355m (A) and 5050m (B) against Sherpa at 5050m (C). Apnea at altitude caused prolonged burst periods and loss of characteristic burst "peak" in Lowlanders . However, these prolonged burst remained contained with a cardiac cycle. Sherpa did not develop prolonged burst firing patterns at 5050m.

**FIGURE 2:** Integral burst area (delta % change relative to baseline, mean± SD; denoted as "#A" ) and incidence of bursts (% of individuals who exhibited a burst during the respective cardiac cycle, denoted as "#B") within Lowlanders (n.=14) and Sherpa (n=8) during apnea. Values represent 10 cardiac cycles prior to volitional breakpoint (indicated by dashed red line along with mean apnea duration). *Panel 1,2*, Lowlanders at 344m (blue) and 5050m (orange), *Panel 3,* Sherpa at 5050m (red). Maximum integrated burst area was determined as the highest response during the 10 cardiac cycles for each participant. Burst incidence was calculated as the number of individuals (represented as a %) exhibiting a burst during that respective cardiac cycle. Lowlanders showed an increase in sympathetic activity prior to apnea breakpoint at both low and high altitude ($P<0.05$). Multiple comparisons were assessed for integral burst area using pre-planned contrasts of Lowlanders from low to high altitude (paired t-tests), and Lowlanders to Sherpa at high altitude (unpaired t-tests) with an adjusted alpha value corrected for multiple comparisons. Sherpa exhibited a smaller increase in burst area ($P<0.05$) versus Lowlanders at 5050m. The incidence of bursts in Lowlanders prior to volitional breakpoint was 100% while

incidence of bursts in Sherpa was 88%. * Significant difference from respective baseline P<0.05; † Significantly different from Lowlanders at high altitude, P<0.05.

**FIGURE 3:** Bar graph (Mean± SD) representing the absolute changes from baseline in normalized total peak SNA (au/min) and change in peak mean arterial pressure (mmHg) during apnea. The normalized SNA represents the sum of SNA across the duration of 10 cardiac cycles. The change in peak mean arterial pressure represents the change in peak blood pressure immediately following (between 0-15 seconds) apnea breakpoint. Statistical analysis for normalized total peak SNA and change in mean arterial pressure was performed via one-way ANOVA between Lowlanders at low altitude, Lowlanders at High Altitude. All groups exhibited a significant increase in MSNA and blood pressure. However, acclimatized Lowlanders (5050m) and Sherpa (5050m) exhibited a smaller change in total SNA compared to Lowlanders at low altitude (334m). Acclimatized Lowlanders (5050m) and Sherpa (5050m) also had smaller blood pressure response compared to Lowlanders at low altitude (334m). * Significant increase with respect to baseline, P<0.05; † Significantly different from other groups, P<0.05, ‡ Significantly different from Lowlanders at 334m only, P<0.05.

**FIGURE 4:** Line graph representing absolute burst frequency (Denoted as "A"; bursts/min), incidence, (Denoted as "B"; bursts / 100 cardiac cycles) and total sympathetic activity (Denoted as "C"; au) in Lowlanders at low altitude (n=14; white circle), high altitude (n=14; black circle), and Sherpa at high altitude (n=6; black triangle) during the isometric handgrip and occlusion protocol. Burst frequency, incidence, and total SNA during baseline, IHG, and PECO protocols, a two-way repeated measures ANOVA compared the main and interaction effects in the distributions between conditions.Lowlanders at exhibited both an interactive and main effect in

503 burst Frequency (P<0.001), and incidence (P<0.001). Sherpa exhibited an overall lower burst
504 frequency, incidence and total MSNA during the BL, IHG and PECO compared to acclimatized
505 Lowlanders (each P<0.001). During IHG burst frequency (all P<0.001), burst incidence (all
506 P<0.001) and total MSNA (all P<0.001) were elevated significantly in Lowlanders at 344m and
507 5050m, and Sherpa at 5050m respectively. No further increase in burst frequency or total MSNA
508 occurred between IHG and PECO, although burst incidence climbed due to the concurrent return
509 of heart rate to baseline during PECO (Figure 7). The increase in MSNA occurring with
510 IHG/PECO was not different between groups.
511
512 **FIGURE 5.** Line graph representing absolute Heart Rate (Denoted as "A"; bpm), Mean
513 Arterial Pressure, (Denoted as "B"; mmHg) and Total Peripheral Resistance (Denoted as "C";
514 mmHg/L/min) in Lowlanders at low altitude (n=14; white circle), high altitude (n=14; black
515 circle), and Sherpa at high altitude (n=6; black triangle) during the isometric handgrip and
516 occlusion protocol. Heart rate, mean arterial pressure, and total peripheral resistance during
517 baseline, IHG, and PECO protocols, a two-way repeated measures ANOVA compared the main
518 and interaction effects in the distributions between conditions. While Sherpa tended to have an
519 overall lower blood pressure (P<0.05), the absolute change in blood pressure response to IHG
520 and PECO were similar between Sherpa (+15± 4 and +12± 6 mmHg) and Lowlanders (+15± 6
521 and +12± 8 mmHg) at 5050m (Figure 5). The change in total peripheral resistance was also
522 similar between Sherpa (+2.6± 2.8 and +2.9± 2.9 mmHg/L/min) and Lowlanders (-0.7± 1.9 and
523 +0.6± 1.7 mmHg/L/min) at 5050m . Thus, unlike the apnea protocol we did not detect
524 significant differences in neurovascular reactivity during IHG / PECO stress with altitude (in
525 Lowlanders) or between groups (at 5050m).
526

**Figure 6.** Line graph representing absolute Brachial Artery Flow (Denoted as "A"; mL/min), Brachial Artery Resistance , (Denoted as "B"; mmHg/mL/min) and Brachial Artery Conductance (Denoted as "C";mmHg/L/min) in Lowlanders at low altitude (n=8; white circle), high altitude (n=8; black circle), and Sherpa at high altitude (n=4; black triangle) during the isometric handgrip and occlusion protocol. All vascular responses during baseline, IHG, and PECO protocols, a two-way repeated measures ANOVA compared the main and interaction effects in the distributions between conditions. Basal forearm blood flow was not different between Lowlanders at 344m (46± 39 mL/min), 5050m (22± 42 mL/min), or Sherpa at 5050m (56± 24 ml/min). Both IHG and PECO saw no change in contralateral brachial blood flow for Sherpa (+29± 25 and +12± 8 L/min) and Lowlanders (+8± 23 and +2± 23 L/min) at 5050m. The HG and PECO protocols did not result in a change in contralateral brachial resistance for Sherpa (-0.3± 1.1 and -0.9± 1.9 mmHg/mL/min) and Lowlanders (-0.6± 1.0 and -0.2± 0.9 mmHg/mL/min) at 5050m. There was a significant main effect for brachial artery conductance ($P<0.05$), with Lowlanders having a higher brachial artery conductance at altitude than Sherpa , though the absolute change during IHG and PECO were similar between Sherpa (-0.1± 0.3 and -0.1± 0.4 mmHg/mL/min) and Lowlanders (+0.3± 0.3 and +0.5± 0.5 mmHg/mL/min) at 5050m.

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664

**TABLE 1:** Participant demographics and metrics of basal neuro-cardiovascular function in lowlanders (at 344m and 5050m) and Sherpa (at 5050m).

| | LOWLANDERS | | SHERPA |
|---|---|---|---|
| | 344m (N = 14) | 5050m (N = 14) | 5050m (N = 8) |
| ***Subject Demographics*** | | | |
| Age (years) | 27±6 | 27± 6 | 32±13 |
| Height (m) | 1.77±0.8 | 1.77± 0.08 | 1.68±0.08 |
| Weight (kg) | 72.2±10.1 | 69.4± 8.6 | 63.7±10.1 |
| BMI ($kg/m^2$) | 23.1±2.8 | 22.2±2.5 | 22.8±3.5 |
| ***Resting Cardiovascular Function*** | | | |
| Heart Rate (bpm) | 61 ± 15 | 70 ± 15* | 71± 5 |
| $SPO_2$ (%) | 98 ± 1 | 83 ± 3* | 83 ± 4 |
| Systolic Pressure (mmHg) | 119 ± 9 | 113 ± 13 | 111 ± 9 |
| Diastolic Pressure (mmHg) | 66 ± 7 | 70 ± 10 | 65 ± 8 |
| Mean Pressure (mmHg) | 84 ± 8 | 86 ± 11 | 84 ± 9 |
| Cardiac Output (L/min) ♦ | 5.9 ± 1.8 | 5.5 ± 1.4 | 6.0 ± 1.7 |
| Total Peripheral Resistance ♦ | 15 ± 4 | 17 ± 4 | 16 ± 7 |
| ***Resting Sympathetic Function*** | | | |
| Burst Frequency (burst $min^{-1}$) | 11 ± 5 | 30 ± 7* | 23 ± 11*† |
| Burst Incidence (burst 100 $HB^{-1}$) | 25 ± 13 | 53 ± 15* | 30 ± 13*† |
| Burst Amplitude (% of peak) • | 42.1± 22.2 | 46.7±7.9 | 46.3± 19.1 |
| Total Activity (au/min) | 451 ± 206 | 1320 ± 520* | 1103 ± 520* |

♦ Derived from Model Flow calculation.

•Burst amplitude calculated as median and interquartile range

* Significantly different from Lowlanders tested at low altitude (344m); $p < 0.05$.

† Significantly different from Lowlanders tested at high altitude (5050m); $p<0.05$.

**FIGURE 1**

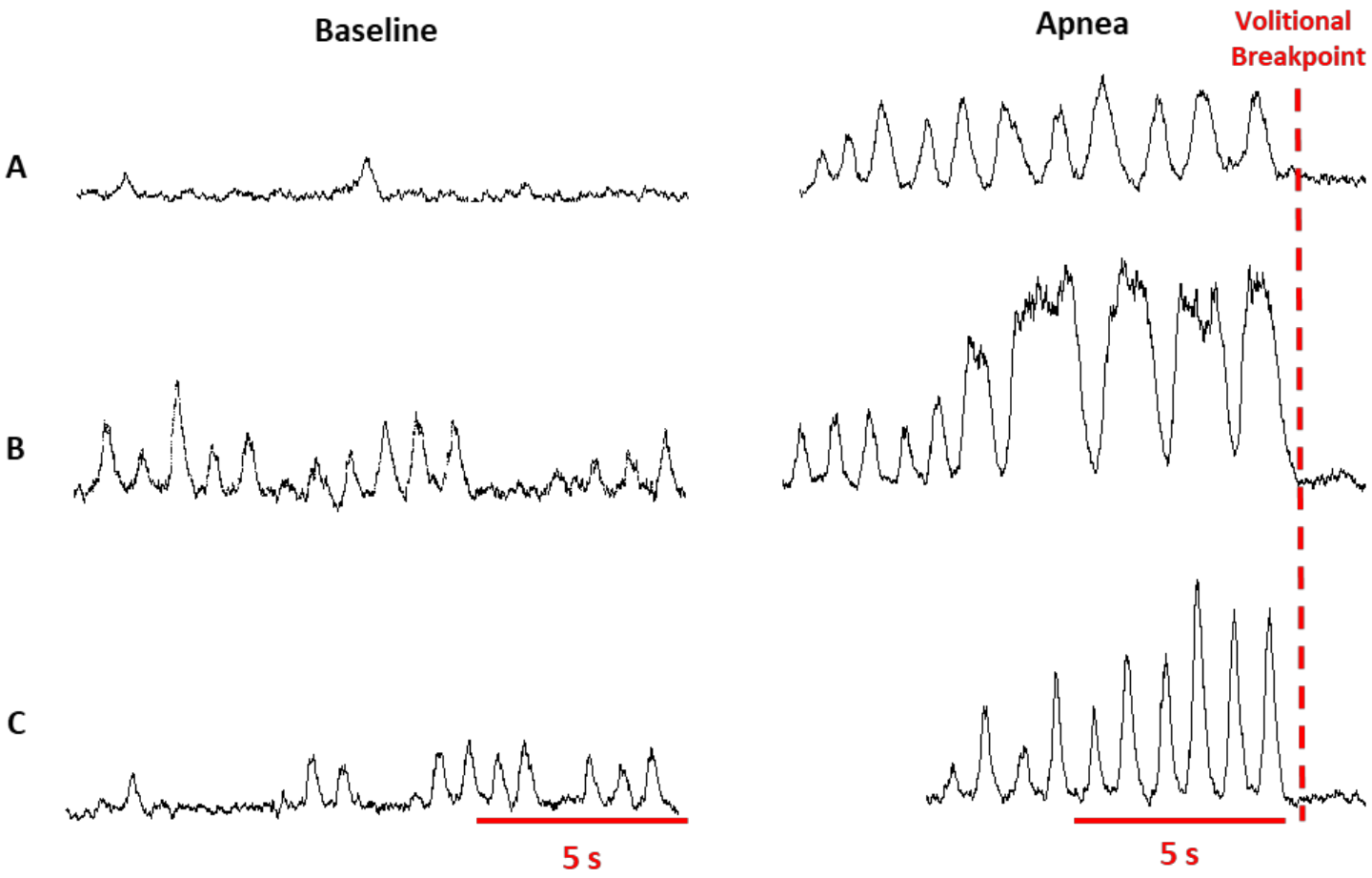
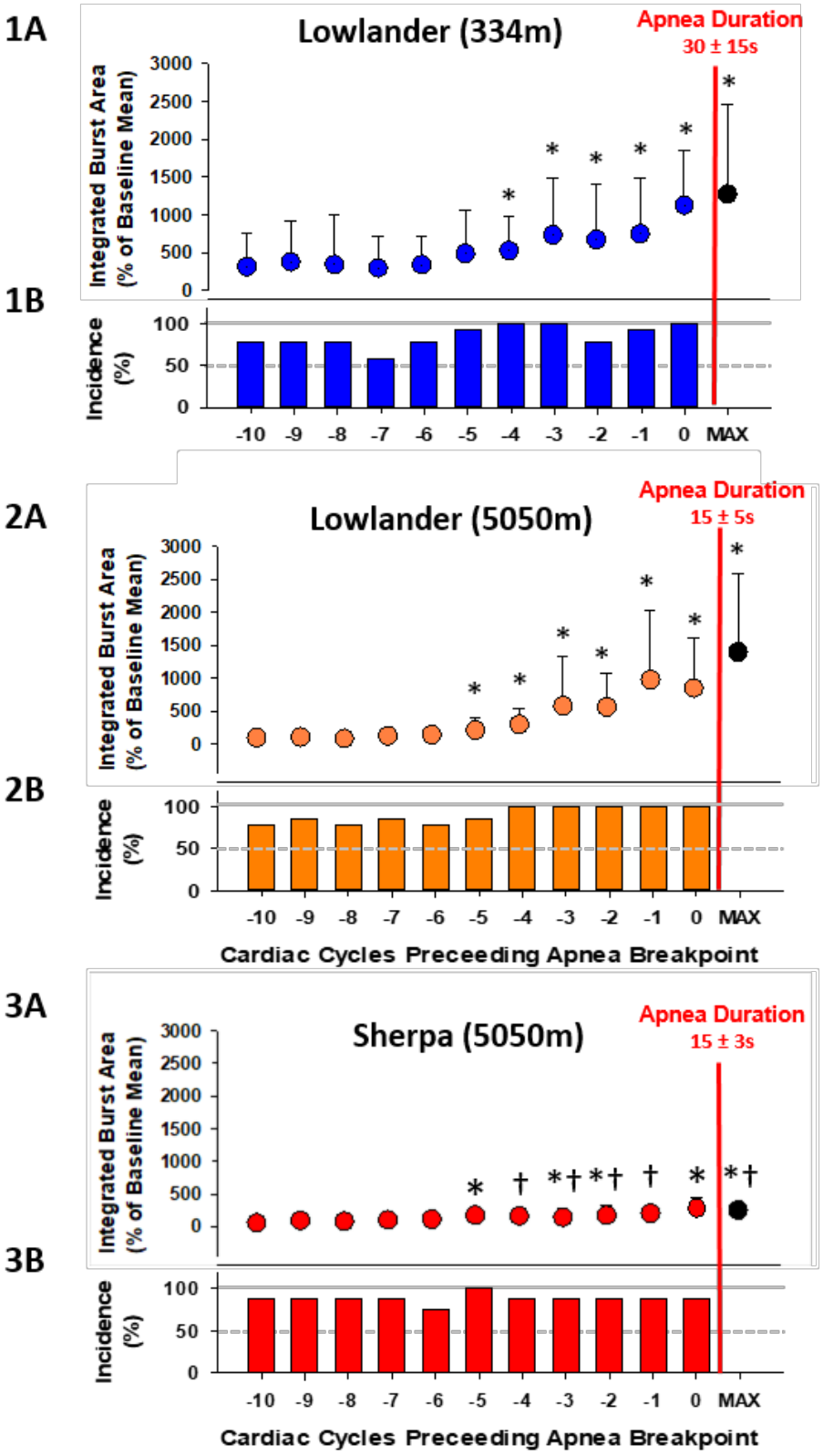


**FIGURE 2**



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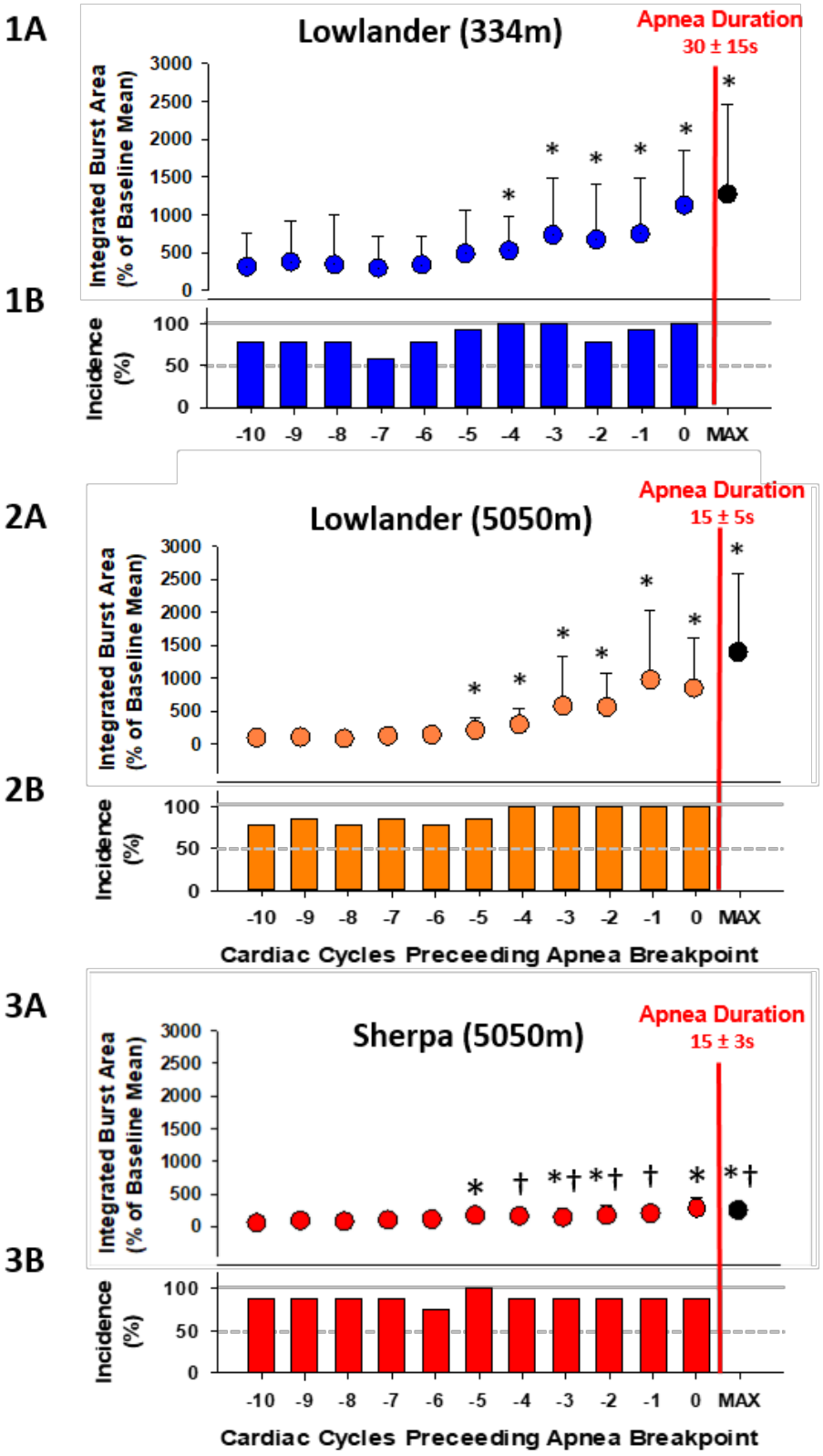

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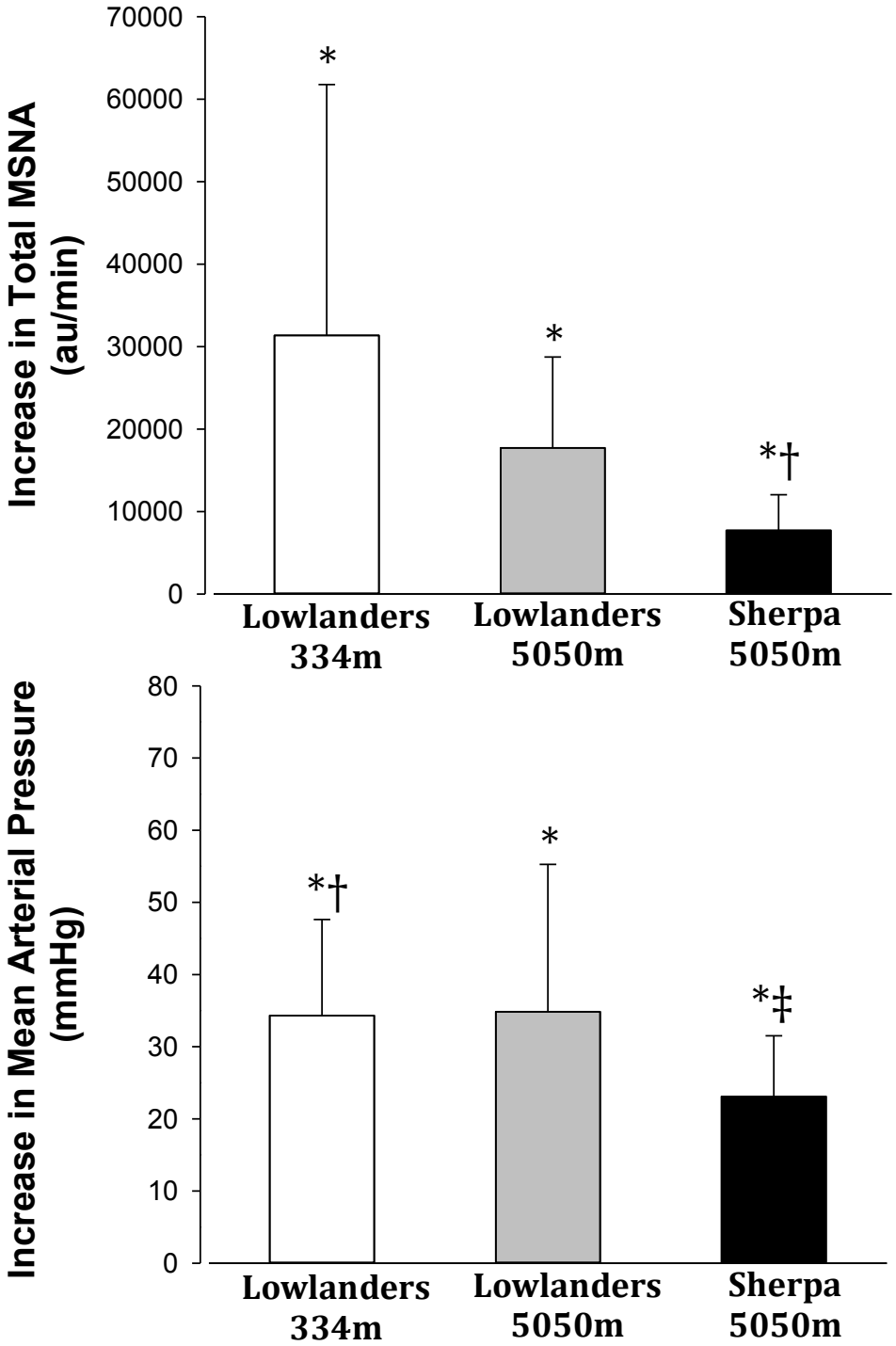

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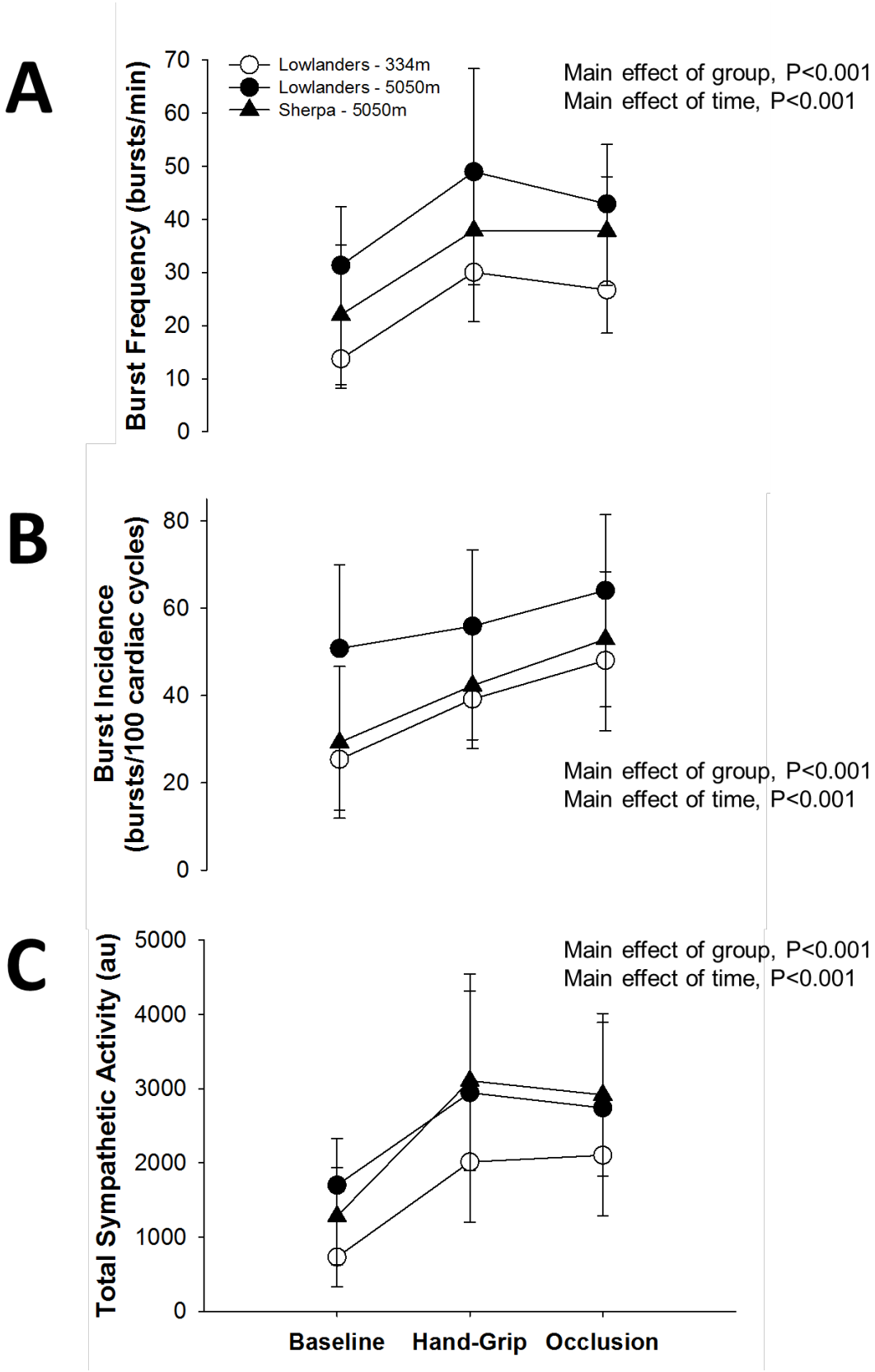

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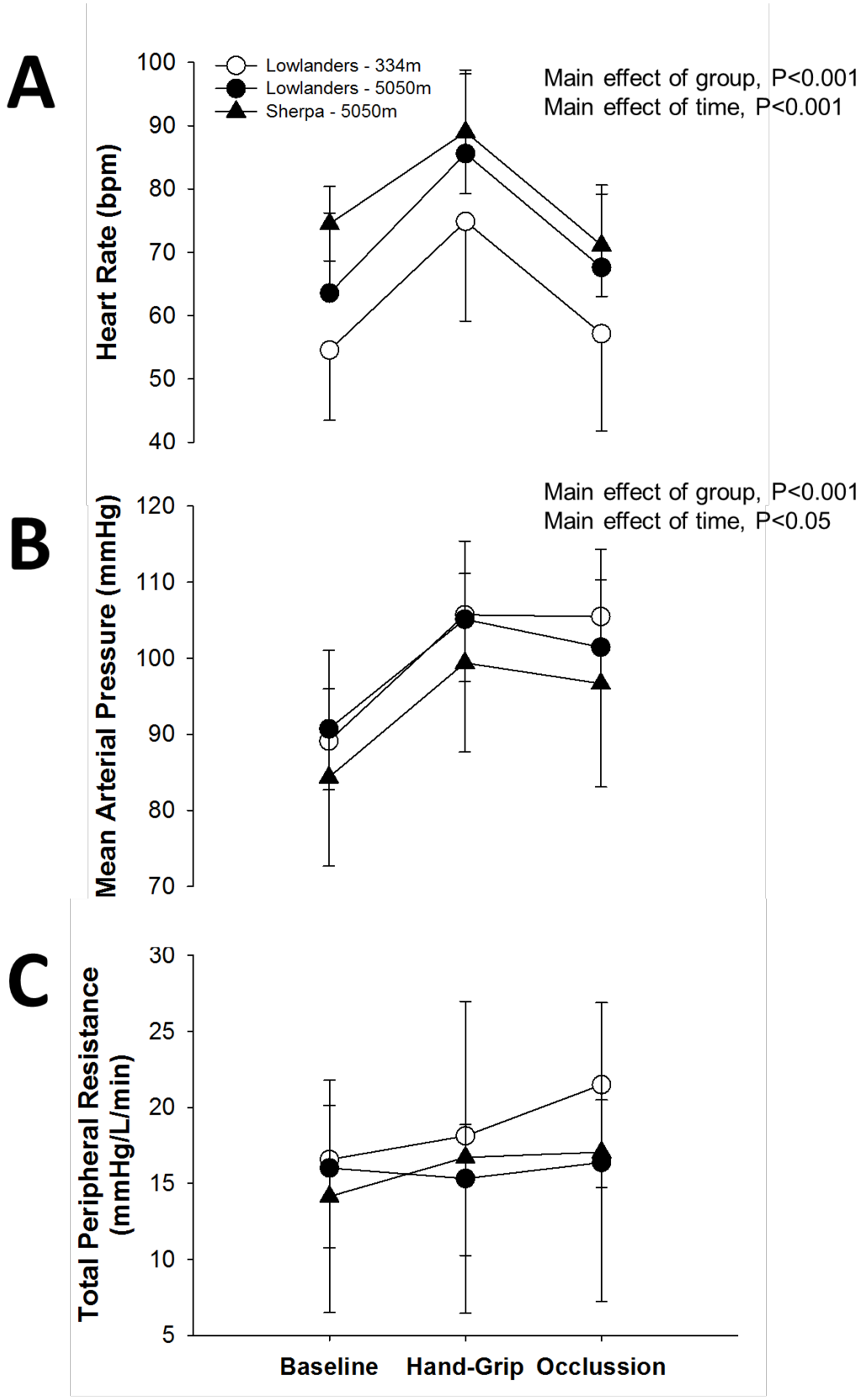
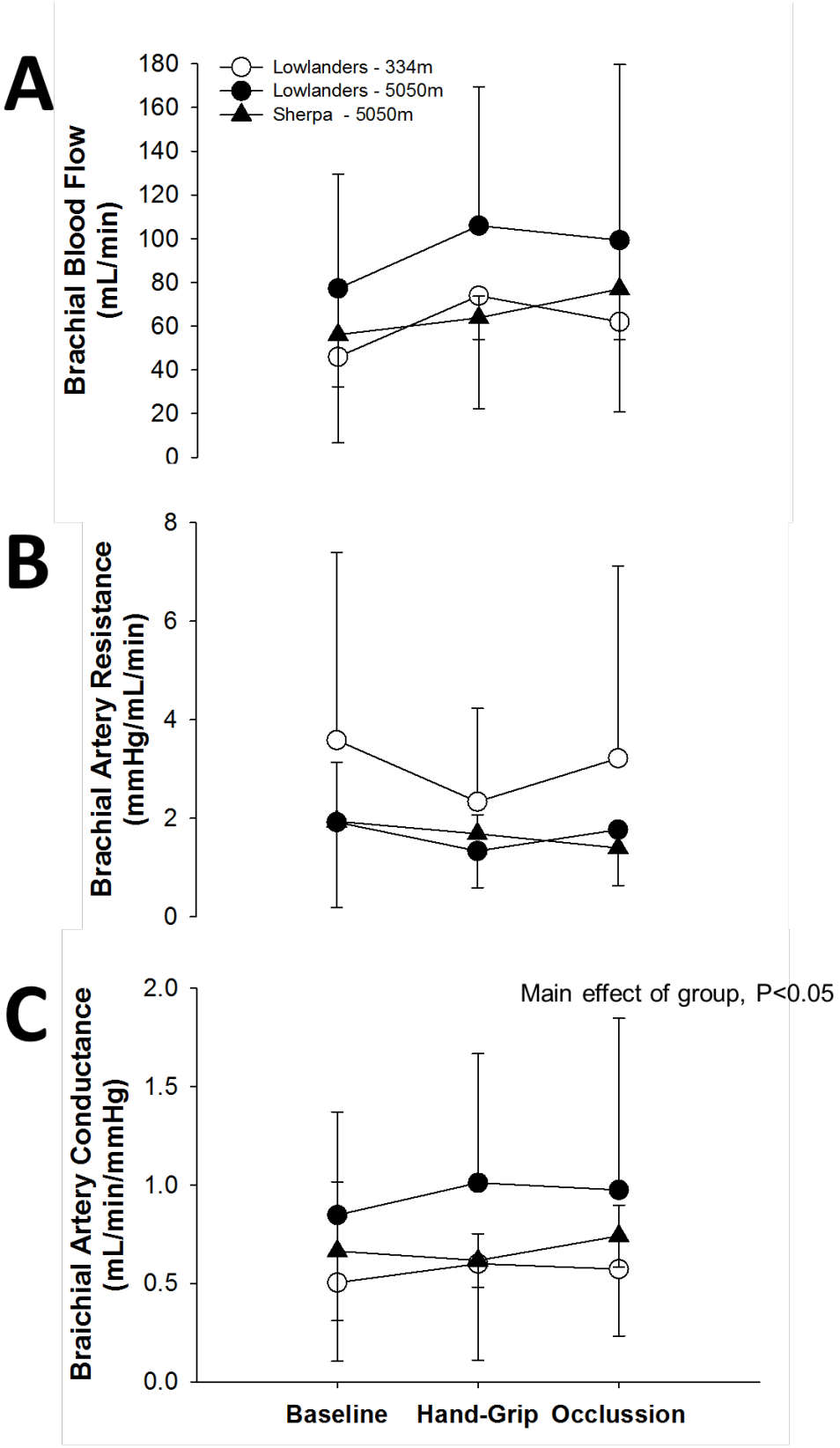


**FIGURE 6**

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